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MiR-31-5p

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Published in:
Allergy

DOI:
[10.1111/all.14060](https://doi.org/10.1111/all.14060)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Final author's version (accepted by publisher, after peer review)

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Tasena, H., Boudewijn, I. M., Faiz, A., Timens, W., Hylkema, M. N., Berg, M., Ten Hacken, N. H. T., Brandsma, C-A., Heijink, I. H., & van den Berge, M. (2020). MiR-31-5p: A shared regulator of chronic mucus hypersecretion in asthma and chronic obstructive pulmonary disease. *Allergy*. <https://doi.org/10.1111/all.14060>

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Article type : Letter to the Editor

MiR-31-5p: a shared regulator of chronic mucus hypersecretion in asthma and chronic obstructive pulmonary disease

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ALL.14060](#)

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Abstract

Chronic mucus hypersecretion (CMH) is prevalent in both asthma and chronic obstructive pulmonary disease (COPD) and is associated with more severe symptoms. Recently, we identified several microRNAs (miRNAs) associated with CMH in COPD. In this study, we extended our analysis to CMH in asthma.

Using small RNA sequencing and RNA sequencing profiles of bronchial biopsies from 65 asthmatic patients, we identified 17 miRNAs associated with CMH in asthma. When comparing them to our previously published dataset in COPD, we found a positive association of miR-31-5p with CMH in both diseases. MiR-31-5p was negatively correlated with multiple predicted targets, including *ST3GAL2*, *PITPNM2*, and *ARHGEF15* which were also associated with CMH. Gene set enrichment analysis revealed the enrichment of *MUC5AC*-associated gene set and CMH-associated gene set in COPD among CMH-associated genes in asthma.

Our findings suggest CMH in asthma and COPD share common regulatory mechanisms mediated, at least in part, by miR-31-5p. This study provides novel insights that could contribute to the development of CMH-targeted therapy that efficiently benefits both asthma and COPD patients.

Keywords

chronic mucus hypersecretion, asthma, COPD, microRNA, miR-31-5p

Word count: 1236

To the Editor:

Chronic mucus hypersecretion (CMH) is prevalent in both asthma¹ and chronic obstructive pulmonary disease (COPD)² and is associated with more severe symptoms.²⁻⁵ Mucus is composed of heavily glycosylated proteins called mucins, of which MUC5AC and MUC5B are the main mucins involved in asthma^{6,7} and COPD.^{8,9} As post-transcriptional regulators of gene expression, microRNAs (miRNAs) play a role in various human diseases.¹⁰ Recently, we identified several miRNAs associated with CMH in COPD.¹¹ In the present study, we extended our analysis to CMH in asthma which is important as it would be efficient to develop the same therapy targeting CMH in both diseases if they share common regulatory mechanisms.

Small RNA sequencing and RNA sequencing was performed on bronchial biopsies from 65 asthmatic patients.¹² The patients either had never been treated with inhaled corticosteroid (ICS), had stopped ICS treatment for 6-8 weeks prior to the bronchoscopy, or were current ICS users. The study approach is illustrated in Fig.1A and detailed methods are described in the Online Repository. Patient characteristics are presented in Table E1.

We first compared miRNA profiles between ICS-naïve asthmatic patients with (n=6) and without CMH (n=14) using linear regression adjusting for age, gender, smoking status and library preparation batch. We identified 17 differentially expressed miRNAs associated with CMH (FDR<.05; Fig.1B and E1). In asthmatic patients with CMH, the expression of miR-31-5p, miR-152-3p, and miR-155-5p was higher, while the expression of miR-15b-3p, miR-15b-5p, miR-16-2-3p, miR-25-3p, miR-92a-3p, miR-106b-3p, miR-185-5p, miR-223-3p, miR-3615, miR-423-5p, miR-425-5p, miR-451a, miR-484 and miR-486-5p was lower compared to patients without CMH. The most strongly up-regulated miRNA was miR-31-5p (4.11 fold) and the most strongly down-regulated one was miR-486-5p (9.47 fold) (Table E2). Interestingly, miR-31-5p was previously also found to be associated with CMH in COPD, with the same direction of effect.¹¹ When including the current ICS users in the analysis, none of the miRNAs was significantly associated with CMH, reflecting an evident influence of corticosteroids on expression of these miRNAs.

Next, we identified CMH-associated mRNAs with the same approach. Expression of *MUC5AC*, but not *MUC5B*, was significantly higher in the asthmatic patients with versus those without CMH (P<.0001, Fig.1C). When corrected for multiple tests (FDR<.05), we identified 2 mRNAs associated with CMH, i.e. *Chromosome 3 Open Reading Frame 70 (C3orf70)* and *phosphatidylinositol transfer protein membrane associated 2 (PITPNM2)*. Using Gene Set Enrichment Analysis (GSEA), we assessed whether MUC5AC-associated core genes¹³ and CMH-

associated genes in COPD¹¹ were enriched among the genome-wide mRNAs, ranked based on the strength (fold change) of their association with CMH in ICS-naïve asthma patients as determined by linear regression. As expected, we found significant enrichment ($P<.001$) of both gene sets among the ranked list (Fig.1C-1D), suggesting that MUC5AC is associated with CMH and that CMH in asthma and COPD share common regulatory mechanisms.

We then assessed whether any of the CMH-associated miRNAs were correlated with MUC5AC or MUC5B using matched small RNA-sequencing and RNA-sequencing profiles in all asthmatic patients ($n=65$) (Table E3). There was a significant positive correlation between miR-16-2-3p and MUC5B ($p=0.02$) and a trend of positive correlation between miR-31-5p and MUC5AC ($p=0.06$). Similar to asthma, miR-31-5p was not correlated with MUC5B in the COPD dataset. MUC5AC expression was not measured by the microarray we used for the COPD dataset (Affymetrix HuGene ST1.0v1), however, we previously showed that MUC5AC-associated core genes were enriched among miR-31-5p-correlated genes¹¹ suggesting that miR-31-5p regulates CMH, at least in part, via modulation of MUC5AC synthesis.

Since we found miR-31-5p to be associated with CMH in both asthma and COPD, we assessed correlations between miR-31-5p and genome-wide mRNA expression regardless of their association with CMH using matched small RNA-sequencing and RNA-sequencing profiles in all asthmatic patients ($n=65$). As miRNAs inhibit translation of their mRNA targets partly by inducing mRNA degradation, we expected the mRNA targets of miR-31-5p to be negatively correlated with this miRNA. Spearman's correlation coefficient revealed 890 genes of which expression levels were negatively correlated with miR-31-5p ($FDR<.05$). Among these 890 negatively correlated genes, 48 were predicted miR-31-5p targets according to TargetScan or miRDB or both (Fig.2A). Next, we applied the same approach on our previously published COPD bronchial biopsy dataset¹¹ which resulted in 62 predicted targets negatively correlated with miR-31-5p (Fig.2A). When combining these 48 and 62 predicted targets, we found 17 overlapping negatively correlated predicted targets of miR-31-5p in asthma and COPD (Fig.2A, Table E4). To identify common mechanisms in which these 17 genes may be involved, we performed GO Enrichment Analysis^{14,15} but found no significant enrichment of GO terms.

Interestingly, of these 17 miR-31-5p correlated targets, *ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 2 (ST3GAL2)*, *PITPNM2* and *Rho guanine nucleotide exchange factor 15 (ARHGEF15)* showed significantly lower expression levels in patients with CMH compared to those without CMH in both diseases (Fig.2A and B). When focusing on ICS-naïve group alone,

we found that the negative correlation between miR-31-5p and ARHGEF15 remained nominally significant ($p=.03$), while there was a trend for a negative correlation with ST3GAL2 ($p=.05$) and PITPNM2 ($p=.08$). *ST3GAL2* is a member of sialyltransferases. Previous studies showed that higher sialylation of airway mucins is present in bacterial infected patients with cystic fibrosis and with chronic bronchitis compared to non-infected patients.¹⁶ This sialylation, occurring during mucin biosynthesis, could influence host-microbial interaction during infection.¹⁷ Thus, *ST3GAL2* may play a role in mucin glycosylation and it is worthwhile to investigate if and how this contributes to CMH. Little is known about the function of *PITPNM2*, while *ARHGEF15* is a Rho guanine nucleotide exchange factor specifically expressed in endothelial cells.¹⁸ Notably, all these 17 genes are expressed in both human-derived basal epithelial cells and goblet cells, as assessed in our single-cell RNA sequencing profiles of bronchial brushes from 6 non-asthmatic control donors and 6 asthmatic donors (BioRxiv 527408).¹⁹

It is not yet conclusive whether higher miR-31-5p expression in CMH is a reflection of its stimulatory or protective role on mucus production. Previously, miR-31-5p expression was found to be slightly lower in asthmatic airway epithelial brushings compared to those of healthy controls but CMH status of these patients was not reported.²⁰ Although bronchial biopsies contain various cell types apart from epithelial cells, we found no difference in numbers of inflammatory cells, i.e. T-cells, B-cells, neutrophils, macrophages, eosinophils, and mast cells, between the asthmatic patients with and without CMH. This suggests that these findings were not driven by infiltration of different inflammatory cells.¹² As our data is based on miRNA-mRNA correlations, future studies are warranted to confirm the direct interaction and functional consequences. Furthermore, these miRNAs and mRNAs were identified using a relatively small sample size and the patients with CMH were all males (Table E1). Therefore, similar analyses performed on a larger cohort with equal gender distribution would strengthen these findings. Since these findings are based on association analyses, future functional studies are required to elucidate whether and how these genes and miR-31-5p directly contribute to CMH. Besides, we encourage other researchers who could to replicate these findings using other measurements of CMH, such as overall mucin concentration in sputum which is one of the important markers of CMH.⁹

This is the first study to propose miR-31-5p as a shared regulator of CMH in both asthma and COPD. We identified several mRNAs potentially targeted by miR-31-5p, including *ST3GAL2*, *PITPNM2*, and *ARHGEF15* that were negatively associated with CMH in both diseases. These findings provide novel and important insights on common CMH regulatory mechanisms and could

contribute to the development of CMH-targeted therapy that efficiently benefits both asthma and COPD patients.

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Acknowledgement of funding

We thank the Dutch Lung Foundation and GlaxoSmithKline plc (GSK) for funding this study and we thank Martijn Nawijn for his support on the analysis of the single-cell RNA sequencing dataset.

A Conflict of Interest disclosure statement

HT, IMB, AF, WT, MNH, MB, NHTtH, CAB, and IHH report no conflict of interest. MvdB received a research grant for this study from GSK and for other studies from TEVA, Chiesi, Astra Zeneca, and Genentech.

A statement of contribution

HT, AF, WT, MNH, CAB, IHH and MvdB contributed to the study concept and design. NHTtH and MvdB coordinated patient inclusion and data collection. NHTtH performed bronchoscopy. MvdB secured funding for the study. MvdB and IMB organized and performed the RNA- and small RNA-sequencing. MB performed the analysis on the single-cell RNA sequencing data. HT, IMB, CAB, IHH and MvdB analysed and interpreted data. HT drafted the manuscript under

supervision of CAB, IHH and MvdB. HT, AF, WT, MNH, CAB, IHH and MvdB critically read and revised the manuscript. All authors have read, reviewed and approved the final manuscript.

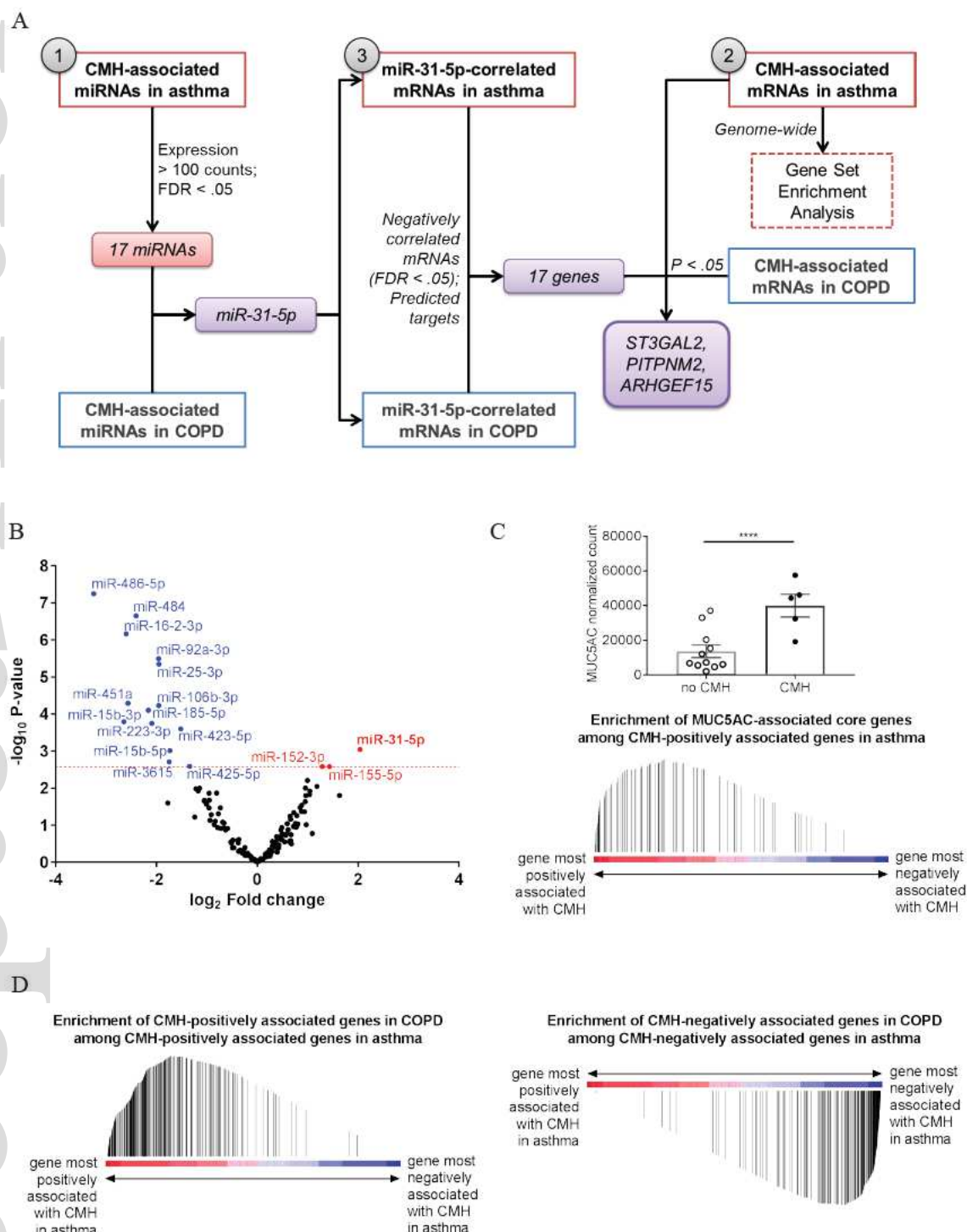


FIGURE 1. MiRNAs and associated genes potentially regulate CMH. A, An overview of the study approach. B, MiRNAs differentially expressed with CMH in asthma. Linear regression was

performed on a group of ICS-naïve asthmatic patients (n=20). Only miRNAs of >100 counts were shown. Dot line indicates a significance of $FDR < .05$. C, Enrichment of MUC5AC-associated genes among CMH-positively associated genes ($P < .001$) and higher MUC5AC expression in asthmatic patients with CMH vs without CMH. **** $P < .0001$. D, Enrichment of CMH-associated genes in COPD among CMH-associated genes in asthma ($P < .001$). Height of each bar in the enrichment plot represents the enrichment score of each gene in the gene set of interest.

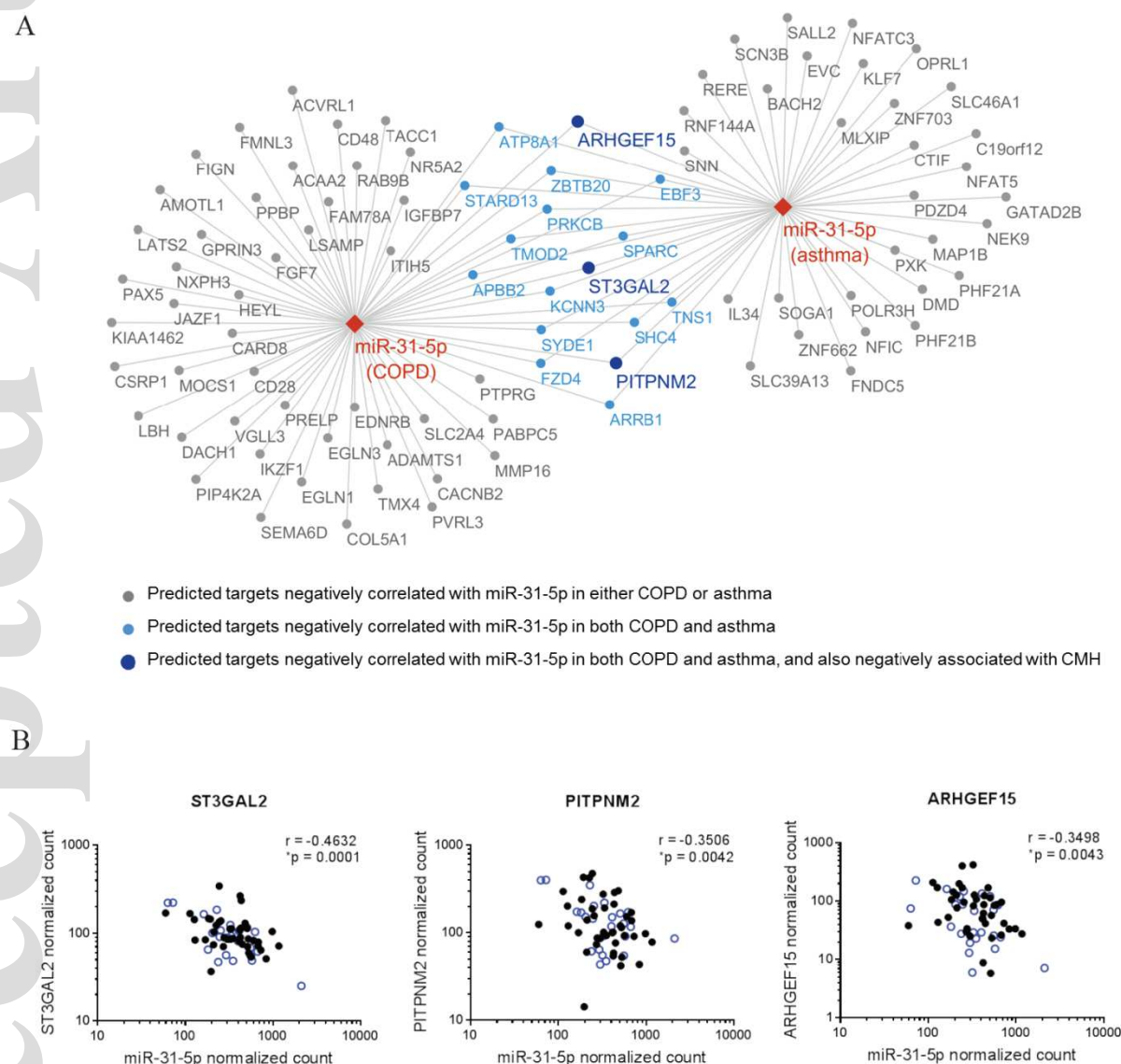


FIGURE 2. Shared mechanisms of CMH in asthma and COPD likely modulated by miR-31-5p. A, MiR-31-5p-negatively correlated predicted targets. B, Correlations of miR-31-5p and its CMH-

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associated targets in all asthmatic patients (n=65). Closed circles represent expression in patients who have been or are currently treated with ICS. Open circles represent expression in ICS-naïve group. Statistics shown in the plots (r and p values) were based on all patients. Spearman correlation was performed using R (v3.2.5).

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